POLLEN MORPHOLOGY IN THE JUGLANDACEAE, I: POLLEN SIZE AND PORE NUMBER VARIATION

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ALTHOUGH IDENTIFICATION OF fossil pollen and spores to the family or generic level provides valuable paleoecological data, it is obvious that specific identifications are to be desired. Unfortunately in many taxa there are no distinctive morphological features which can be used to effect such identifications, although in some difficult groups measurable characters such as pollen size or pore number have been utilized. Examples of this are the use of pollen size in *Pinus* (Cain 1940, 1944, Cain and Cain 1944, 1948a, 1948b, Buell 1946a, 1946b, Hansen 1947) and *Betula* (Leopold 1956) and the use of pore number in *Alnus* (Leopold 1955). It has been emphasized repeatedly (e.g., Christensen 1946, Faegri and Iversen 1950, and Whitehead 1962) that the use of such criteria is beset with many difficulties. First of all, one must have adequate knowledge of the range of variation displayed by the character selected. This can be obtained only by accumulating data from a large number of collections selected to cover the geographic range of the species and the spectrum of ecological conditions in which it occurs. For pollen size frequency studies one must also assess with care the effect of chemical treatment, mounting medium, and environment of preservation.

As yet the statistical approach has not been applied to members of the Juglandaceae, even though studies of Pleistocene and Tertiary sediments have suggested the possibility of separating species of *Carya* on the basis of pollen size and species of *Juglans* on the basis of pore number. For example, it is readily apparent from studying any sample which contains much *Carya* pollen that there is a considerable size range for the grains. This suggests that there might be distinct size differences among the various species. Similarly, work with Pleistocene material in the east has indicated the possibility of separating grains of *Juglans cinerea* L. from those of *J. nigra* L. on the basis of pore number. Grains of the former appear to have fewer pores. However, adequate data on pollen size in the extant species of *Carya* and pore number variation in the aforementioned species of *Juglans* have not been available.

In the present paper the results of a study of the pollen size-frequency characteristics of *Carya tomentosa* Nutt. and *C. cordiformis* (Wang.) K. Koch will be described. These two species were selected so that extensive data would be available for at least one species from each section of the genus (*C. tomentosa* from section CARYA (sect. *Eucarya* C. DC.) and *C. cordiformis* from section APOCARYA C. DC.). In addition, preliminary size frequency data for several other species of *Carya* will be discussed.

Lastly, the results of a detailed study of pore number variation in *Juglans cinerea* and *J. nigra* will be presented and the reliability of this criterion for specific identifications discussed.

The author wishes to express his appreciation to Dr. Elso S. Barghoorn, under whose thoughtful direction the variational study was initiated, and to the Jersey Production Research Company, Tulsa, Oklahoma, for permission to publish the results of the size study which was carried out as part of a general survey of pollen morphology in the Juglandaceae while the writer was a consultant during the summer of 1960. Grant G-17277 from the National Science Foundation has provided support for the final phase of the project.

1. POLLEN SIZE VARIATION IN CARYA TOMENTOSA AND C. CORDIFORMIS

Eleven collections each of *Carya tomentosa* and *C. cordiformis* were used for the size variation study. Between 1 and 3 collections have been used for study of the other species of *Carya* examined. The specimens studied are cited at the end of this paper.

Pollen of *Carya* was prepared according to the following technique:

1. Boil in KOH for 3 minutes.
2. Wash twice with distilled water.
3. Wash once with glacial acetic acid.
4. Acetolyze for 1 minute (10:1, acetic anhydride: concentrated sulfuric acid).
5. Wash twice with distilled water.
6. Wash once with 95% alcohol.
7. Wash twice with absolute alcohol.
8. Wash once with benzene (U.S.P., thiophene free).
9. Mount in silicone oil (Dow Corning 200 Fluid, viscosity 12,500 centistokes).

Use of the above preparation and of silicone oil for the size study was predicated by the considerations outlined by Christensen (1946, 1954), Andersen (1960), and Whitehead (1961, 1962).

Size measurements were carried out under high dry magnification utilizing an ocular interval of 2.04 micra. One hundred grains were measured for each collection of *Carya tomentosa* and *C. cordiformis*, and 50 grains for the collections of the other species. The linear dimension selected for measurement is illustrated in FIGURE 4.

The results of the pollen size study are presented in FIGURE 1, and in TABLES 1, 2, and 3. It is clear that there is considerable variation in pollen size in *Carya*.

Grains of *Carya cordiformis* range in size from 26.52 μ to 46. 92 μ, with a mean of 40.36 $\pm$ 2.89 μ. The modal class for the entire population is 40.80 μ. For the individual collections the modal class varies from 34.68 μ to 42.84 μ. The pollen grains of *C. tomentosa* vary in size from 40.80 μ to 63.24 μ, with a mean of 50.65 $\pm$ 3.41 μ. The modal class is

51.00 μ. Modes for the individual collections range from 46.92 μ to 53.04 μ.

Size data on the other species of *Carya* studied are as follows:

Section APOCARYA: *Carya aquatica*, 2 collections, range 36.72–46.92 μ. mean 41.80 μ; *C. illinoensis*, 2 collections, range 38.76–55.08 μ, mean

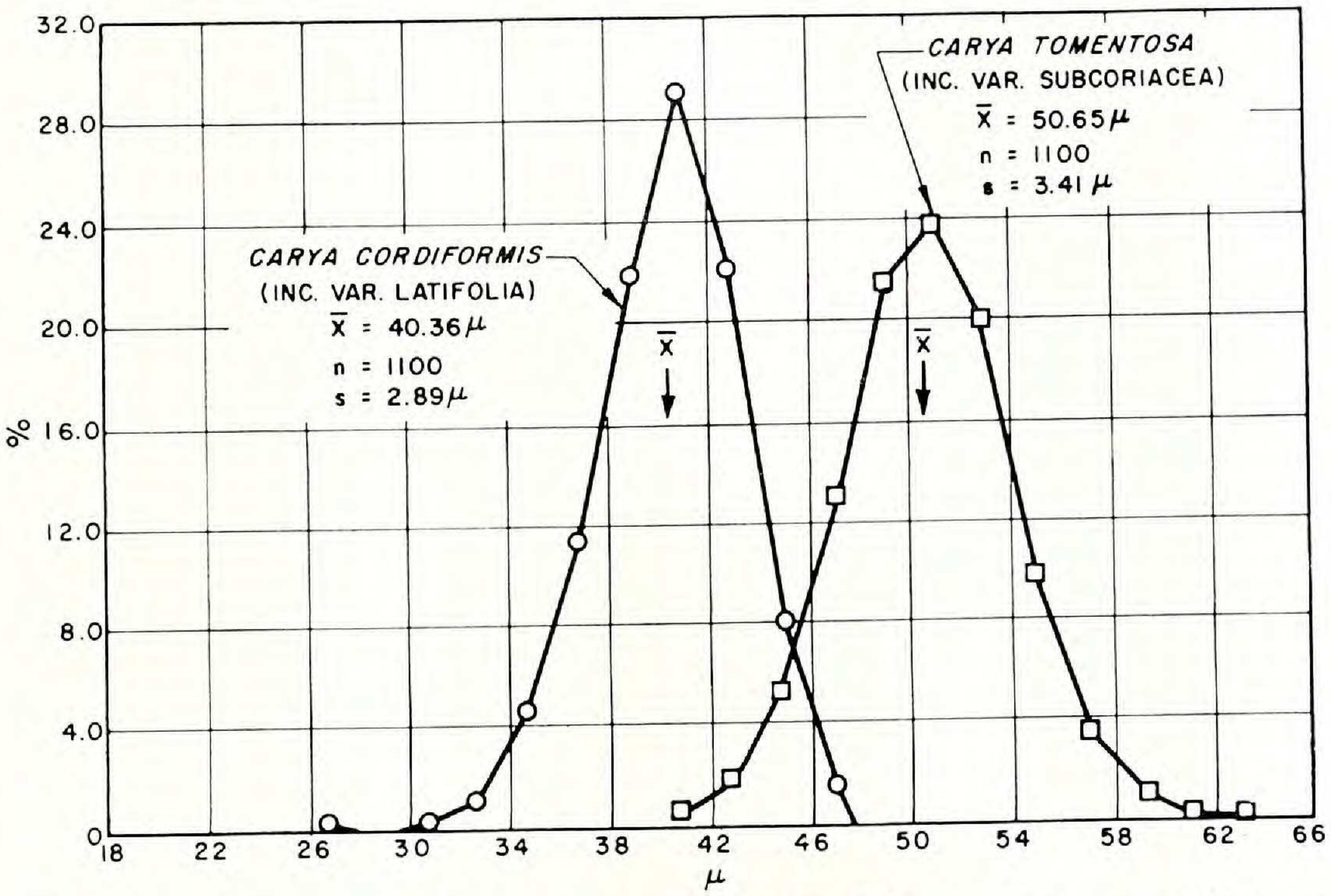


FIG. 1. Pollen size variation in *Carya tomentosa* Nutt. and *Carya cordiformis* (Wang.) K. Koch.

TABLE 1. **Pollen Size Variation in Carya cordiformis**

	SAMPLE NUMBER											
MICRA	1	2	3	4	5	6	7	8	9	10	11	TOTALS
26.52				1								1
28.56				0								0
30.60				2								2
32.64				10		1				1		12
34.68	1	1		42		3	2			3		52
36.72	10	5	4	36	5	17	20	2	2	9	15	125
38.76	38	11	15	7	17	34	38	11	2	24	43	240
40.80	34	38	32	2	32	33	28	34	18	36	32	319
42.84	15	29	31		37	9	11	38	40	23	9	242
44.88	1	13	14		9	3	1	15	28	4	1	89
46.92	1	3	4							10		18
												1100

$\overline{\times}$ = 40.36
s = ±2.89

1, Minnesota; 2, Kentucky; 3, Missouri; 4, South Carolina; 5, Washington, D.C.; 6, New York; 7, Missouri (var. *latifolia*); 8, Oklahoma (var. *latifolia*); 9, Texas; 10, Mississippi; 11, Tennessee.

46.10 μ; *C. myristiciformis*, 2 collections, range 32.64–44.88 μ, mean 39.64 μ.

Section CARYA (sect. *Eucarya* C. DC.) *C. laciniosa*, 2 collections, range 38.76–51.00 μ, mean 45.00 μ; *C. ovata*, 3 collections, range 36.72–51.00 μ, mean 44.93 μ; *C. pallida*, 1 collection, range 42.84–55.08 μ, mean 49.82 μ; *C. glabra*, 2 collections, range 40.80–53.04 μ, mean 46.88 μ; *C. leiodermis*, 1 collection, range 40.80–53.04 μ, mean 47.49 μ; *C. texana*, 2 collections, range 40.80–53.04 μ, mean 46.27 μ; *C. ovalis*, 2 collections, range 36.72–53.04 μ, mean 47.65 μ.

Since only a few measurements are as yet available for most species of *Carya*, it is premature to suggest the possibility of differentiating species on the basis of pollen size. However, the considerable difference in mean pollen size for *C. tomentosa* and *C. cordiformis* (10 micra) and the interspecific differences indicated by the preliminary data on TABLE 2, do suggest that the preparation of a size-frequency curve for fossil material might be instructive. A bimodal curve could then be taken as an indication of the presence of at least 2 species. Further work on pollen size for the other species of *Carya* might then allow one to make tentative suggestions as to the species represented, provided that one has evaluated the variables mentioned previously. Similarly, size frequency analyses of this sort could be utilized for the delimitation of "form species" in pre-Pleistocene sediments.

It is interesting to note that *Carya tomentosa*, like several other species of section CARYA, is a tetraploid, and that *C. cordiformis*, like other species

TABLE 2. **Pollen Size Variation in Carya tomentosa**

	SAMPLE NUMBER											
MICRA	1	2	3	4	5	6	7	8	9	10	11	TOTALS
40.80				3	1		1					5
42.84	1		1	7	5		2		2			18
44.88	5	1	1	28	11	1	7		1		3	58
46.92	20	4	3	38	25	7	21	4	2	2	17	143
48.96	29	20	7	15	40	24	34	10	20	6	32	237
51.00	26	32	18	7	14	31	22	31	30	26	24	261
53.04	13	28	26	2	4	22	10	32	28	41	13	219
55.08	5	12	22			10	3	18	12	17	9	108
57.12	1	3	13			4		4	4	7	2	38
59.16			7			1		1	1	1		11
61.20			1									1
63.24		1										1
												1100

$\overline{X} = 50.648$
$s = \pm 3.41$

1, Texas (var. *subcoriacea*); 2, Indiana (var. *subcoriacea*); 3, Missouri; 4, Pennsylvania; 5, Virginia; 6, Mississippi; 7, Indiana; 8, Georgia; 9, Massachusetts; 10, Oklahoma; 11, Tennessee.

of section APOCARYA, is a diploid (Woodworth 1930, Stone 1961). Since grains of *C. tomentosa* are 10 micra larger on the average than those of *C. cordiformis*, it is possible that pollen size in *Carya* may be correlated with ploidal level. This is true in a number of other taxa (e.g., *Andropogon* (Gould 1957)). Stomatal size shows a similar correlation in *Carya* (Stone 1961). The preliminary size data for the other known tetraploids indicate that mean pollen size (for grains treated with KOH, acetolysis, and mounted in silicone oil) for this ploidal level in *Carya* is above 46 μ (*C. glabra*, 46.88 μ; *C. texana*, 46.27 μ; *C. ovalis*, 47.65 μ), while data for the known diploids suggest that the mean pollen size for that ploidal level is less than 46 μ (*C. aquatica*, 41.80 μ; *C. illinoensis*, 46.10 μ; *C. laciniosa*, 45.00 μ; *C. ovata*, 44.93 μ).

The preliminary data on pollen size for *Carya myristiciformis* (39.64 μ) supports Stone's information on stomatal size, and suggests that this species is a diploid. Similarly, the pollen size data for *C. pallida* (49.82 μ) and *C. leiodermis* (47.49 μ) and Stone's stomatal measurements indicate that these two species are probably tetraploids.

TABLE 3. **Pollen Size Variation in Several Species of Carya**

MICRA	AQUATICA	GLABRA	ILLINOENSIS	LACINIOSA	LEIODERMIS	MYRISTICIFORMIS	OVALIS	OVATA	PALLIDA	TEXANA
	n	n	n	n	n	n	n	n	n	n
26.52										
28.56										
30.60										
32.64						2				
34.68						7				
36.72	4					12	1	2		
38.76	12		1	1		28	1	4		
40.80	30	1	4	12	2	31	1	22		4
42.84	40	5	21	18	3	16	5	22	2	21
44.88	13	26	29	31	9	4	16	36	1	20
46.92	1	40	17	27	12		25	40	6	25
48.96		20	14	9	15		32	22	17	19
51.00		7	10	2	7		15	2	15	10
53.04		1	3		2		4		7	1
55.08			1						2	
57.12										
59.16										
61.20										
63.24										
TOTALS	100	100	100	100	50	100	100	150	50	100
MEAN	41.80 μ	46.88 μ	46.10 μ	45.00 μ	47.49 μ	39.64 μ	47.65 μ	44.93 μ	49.82 μ	46.27 μ

2. PORE NUMBER VARIATION IN JUGLANS CINEREA AND J. NIGRA

Forty-four collections of *Juglans cinerea* and 42 of *J. nigra* have been utilized for the pore number investigation. Pollen of *Juglans* was prepared by acetolysis and mounted in glycerine jelly. The pore number study of *Juglans* was carried out by counting the pores in 100 grains for each collection. Counts were made under high dry magnification by focusing carefully up and down through the individual grains. The problems inherent in making such counts will be apparent in FIGURES 5–10. The results of this study are presented in FIGURE 2.

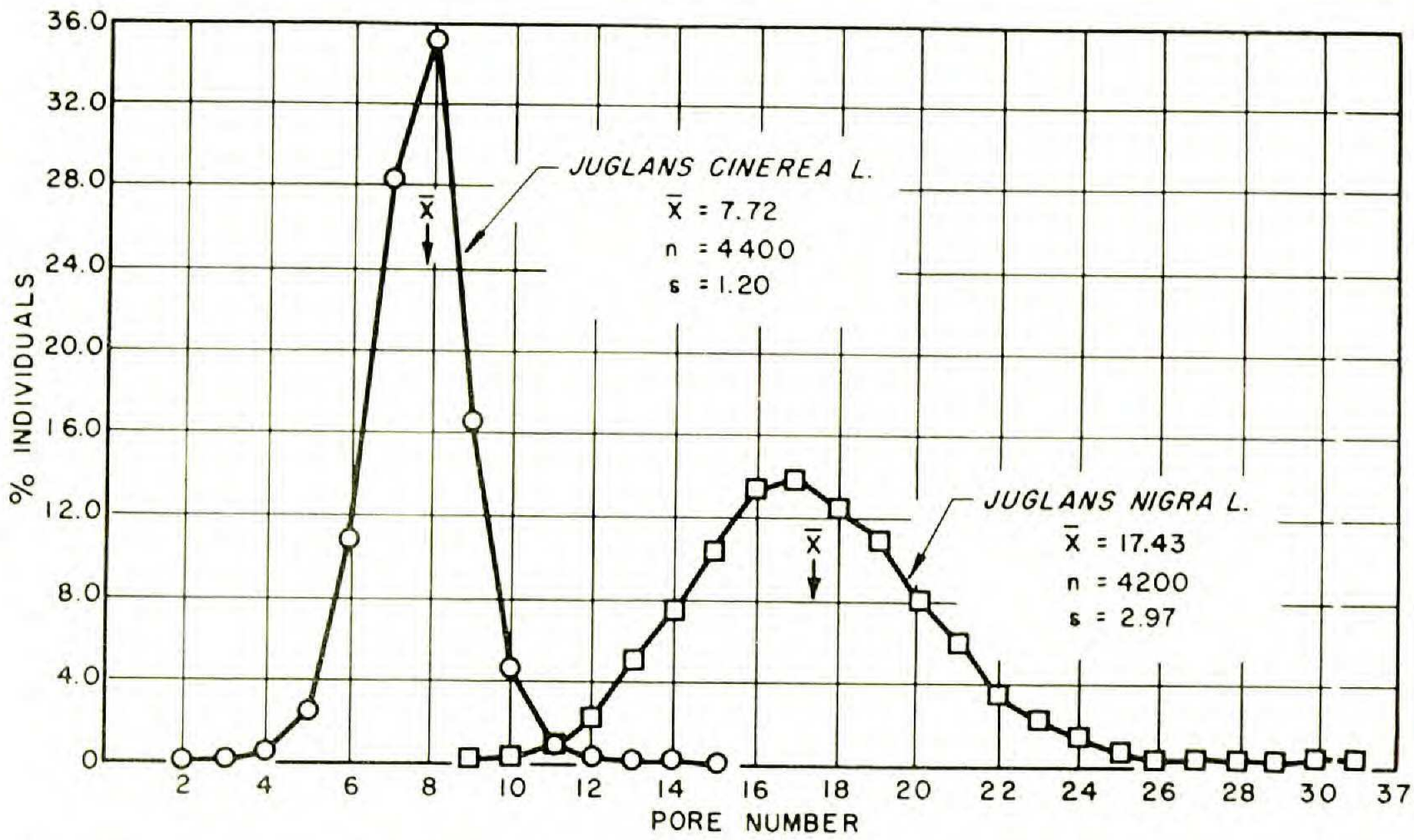
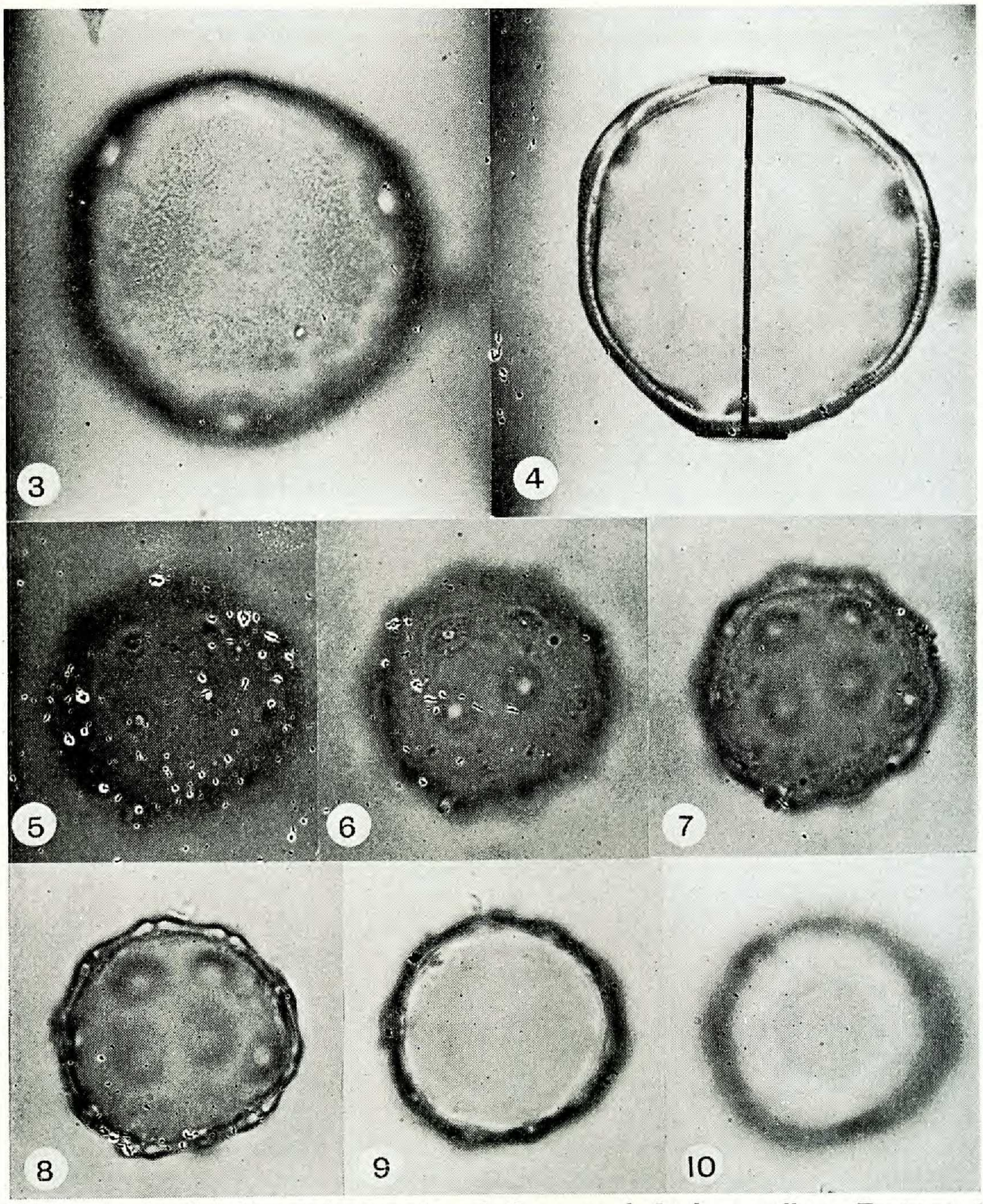


FIG. 2. Pore number variation in *Juglans cinerea* L. and *Juglans nigra* L.

Pore number in *Juglans cinerea* varies from 2 to 15 with a mean of 7.72 ± 1.20. The modal class for the total population is 8 pores. Pore number in *J. nigra* varies from 9 to 37 with a mean of 17.43 ± 2.97. The modal class for the total population is 17. In the individual collections of *J. cinerea* the modal class varies from 6 to 9 pores and in *J. nigra* from 12 to 22.

It is evident that the majority of grains of *Juglans cinerea* can be separated from those of *J. nigra* with reasonable certainty. There is overlap only between 9 and 15 pores, hence grains with less than 9 pores would most certainly be those of *J. cinerea*, and grains with more than 15, *J. nigra*. Grains having 9 and 10 pores are more likely to be those of *J. cinerea*, and grains with from 12 to 15 are more likely to be those of *J. nigra*. Pollen with 11 pores could be assigned with equal probability to either species.

However, one must approach these assumptions with some caution, remembering that the modal class in one collection of *Juglans cinerea*



FIGURES 3–10. Photomicrographs of *Carya* and *Juglans* pollen. FIGURES 3 and 4, *Carya tomentosa* pollen, polar view, two levels of focus. Dimension measured is marked in FIGURE 4. FIGURES 5–10, consecutive levels of focus through a grain of *Juglans nigra*, polar view. Note the heteropolar characteristic of the grain, with the pores being located equatorially and globally, but with one hemisphere pore-free. Magnification 725×.

was as high as 9 and in one collection of *J. nigra* as low as 12. If an entire local population of *J. cinerea* possessed grains with a mean pore number close to 9, then the chances of encountering white walnut grains with from 10 to 15 pores would, of course, be greater. Similarly, if a local population of black walnut possessed grains with a mean pore number close to 12, then the chances of encountering *J. nigra* grains with from 9 to 11 pores would also be greater. In this respect, information on pore

number variation within several local populations of each species would be instructive.

Obviously, the preparation of a pore number frequency curve for fossil material would aid in determining whether only one or both of the species were represented. Unfortunately, one almost never encounters a sufficient number of *Juglans* grains to allow for this. However, noting the number of pores in any other walnut grains encountered in a sample will help suggest which species were present.

SPECIMENS EXAMINED [1]

1. **Carya cordiformis** (Wang.) K. Koch. UNITED STATES. Rhode Island: *Palmer 44337* (A). New York: *Thomas 2152* (A). Minnesota: *Mearns 814* (GH). District of Columbia: *Ward*, 1879 (GH). Kentucky: *Horsey 2321* (A). South Carolina: *Griscom 16503* (GH). Missouri: *Palmer 3943* (A). Mississippi: *Harbison 10* (GH). Texas: *Bush 5588* (A).
2. **Carya cordiformis** (Wang.) K. Koch var. **latifolia** Sarg. UNITED STATES. Missouri: *Palmer 5499* (A). Oklahoma: *Bush 1139* (GH).
3. **Carya tomentosa** Nutt. UNITED STATES. Massachusetts: *Manning*, 1942 (A). Pennsylvania: *Byhouwser & Kobuski 216* (A). Indiana: *Deam 27014* (GH). Virginia: *Allard 2621* (GH). Tennessee: *Ruth 426* (GH). Georgia: *Small*, 1895 (GH). Mississippi: *Harbison 17* (GH). Missouri: *Palmer 5761* (A). Oklahoma: *Palmer 21647* (GH).
4. **Carya tomentosa** Nutt. var. **subcoriacea** Sarg. UNITED STATES. Indiana: *Deam 10182* (A). Texas: *Palmer 7269* (A).
5. **Carya aquatica** Nutt. UNITED STATES. Georgia: *Gillespie 5045* (A). Mississippi: *Harbison 191* (A).
6. **Carya glabra** (Mill.) Sweet. UNITED STATES. Massachusetts: *Faxon*, no date (GH). Georgia: *Harbison 1026* (A).
7. **Carya illinoensis** (Wang.) K. Koch. UNITED STATES. Tennessee: *Gattinger 2565* (GH). Missouri: *Bush 5759* (A).
8. **Carya laciniosa** (Michx. f.) Loud. UNITED STATES. Massachusetts: *Manning*, 1941 (GH). Missouri: *Palmer 39144* (A).
9. **Carya leiodermis** Sarg. UNITED STATES. Arkansas: *Palmer 39298* (A).
10. **Carya myristiciformis** (Michx. f.) Nutt. UNITED STATES. Arkansas: *Letterman*, 1881 (GH). Texas: *Palmer 22438* (A).
11. **Carya ovalis** (Wang.) Sarg. UNITED STATES. Massachusetts: *Manning*, 1935 (GH). Ohio: *Horsey 479* (GH).
12. **Carya ovata** (Mill.) K. Koch. CANADA. Quebec: *Marie-Victorin 28234* (GH). UNITED STATES. North Carolina: *Biltmore Herbarium 1838a* (A). Arkansas: *Palmer 22461* (A).
13. **Carya pallida** (Ashe) Engl. & Graebn. UNITED STATES. North Carolina: *Biltmore Herbarium 5551a* (GH).
14. **Carya texana** Buckl. UNITED STATES. Texas: *Bush 197* (A).
15. **Carya texana** Buckl. var. **arkansana** Sarg. UNITED STATES. Missouri: *Palmer 3928* (GH).

[1] Material of all collections cited was obtained from the herbarium of the Arnold Arboretum (A) or from the Gray Herbarium (GH), Harvard University.

16. **Juglans cinerea** L. CANADA. Quebec: Massawippi, *Jack*, 1914 (A); Ile Perrot, *Marie-Victorin 28230* (GH); Phillipsburg, *Knowlton*, 1925 (GH). Ontario: *Shields*, 1948 (GH). UNITED STATES. Maine: *Narlin*, 1896 (GH). New Hampshire: Jaffrey, *Williams*, 1898 (GH); Lebanon, *Kennedy*, 1894 (GH). Vermont: Charlotte, *Pringle*, 1879 (A); Wallingford, *Williams*, 1908 (GH); Brandon, *Williams*, 1908 (GH). Massachusetts: Waltham, *Williams*, 1897 (GH); Walpole, *Rich*, 1897 (GH); Boxford, *Robinson*, 19?? (A); South Egremont, *C. B. (surname illegible)*, 1919 (A). Connecticut: *Harger*, 1902 (GH). New York: Vaughns, *Burnham*, 1891 (GH); Fall Creek, *Muenscher & Bechtel 665* (A). Ohio: Oberlin, *Kofoid*, 1890 (GH); Friendship, *Demaree 10639* (GH); Columbus, *Horsey*, 1914 (A). Michigan: *W. J. B.*, 1899 (A). Minnesota: *Moore & Thatcher 13015* (A). Wisconsin: *J. H. V.*, 1896 (GH). Iowa: *Pammel 102* (GH). Indiana: *Hill*, 1894 (A). Pennsylvania: Martin's Creek, *Bartram*, 1907 (A); Bradford County, *Byhouwser & Kobuski 212* (A); Bethlehem, *No collector or date listed* (GH); Conestoga River, *Heller*, 1900 (GH). Delaware: *Canby*, 1897 (A). Virginia: Natural Bridge, *Deane*, 1886 (GH); Hot Springs. *Hunnewell 4000* (GH); Marion, *Britton et al.*, 1892 (A). West Virginia: *Harbison 7108* (A). Tennesee: *Palmer 17310* (A). Kentucky: Middleborough, *Horsey 1927* (A); Beattyville, *Horsey 1156* (A). Missouri: Hazelgreen, *Palmer 39201* (A); Booneville, *Palmer 29972* (A); Arcadia, *Palmer 22703* (A); Pontiac. *Bush 13374* (A); Allenton, *Letterman*, 1882 (A); Ralls County, *Davis 1360* (A); Patton, *Kellogg 25860* (A). Arkansas: *Bush 13402* (A).

17. **Juglans nigra** L. UNITED STATES. Rhode Island: *Rehder*, 1927 (A). New York: Tompkins County, *Thomas 2148a* (GH); Queensbury, *Burnham*, 1893 (GH); Hempstead, *Churchill*, 1910 (GH). Pennsylvania: Whitehorse, *Travis 31* (A); Stewartstown, *Adams 4431* (A). Ohio: Gallipolis, *Horsey 2108* (A); Roosevelt Game Preserve, *Demaree 10678* (GH). Michigan: Monroe Lake, *Ehlers 369* (A; GH); Herb. Agricultural College, Michigan, *Beal*, 1899 (A). Illinois: Stark County, *Chase*, 1896 (GH); Havana, *Jones 11231* (GH); Cairo, *Palmer 14920* (A). Indiana: Ingalls, *Smith 5602* (GH); Long Swamp, *Ek*, 1942 (GH). New Jersey: *Long 34600* (GH). Delaware: *Canby*, 1899 (GH). Virginia: Prince George County, *Fernald & Long 11814* (GH); Walker Creek, *Small*, 1892 (A); Gertie, *Fernald & Griscom 4381* (GH). Kentucky: Battlesburg, *Horsey 893* (A); Richmond, *Horsey 1065* (A), Olympia, *Horsey 1714* (A); Mt. Sterling, *Horsey 2307* (A). Tennessee: *Palmer 17541* (A). North Carolina: Granville County, *Faxon*, no date (GH); Biltmore, *Biltmore Herbarium 1314* (GH). Georgia: *Small*, 1895 (A). Missouri: Allenton, *Letterman*, 1882 (A); Joplin, *Palmer 22743* (A); Galena, *Palmer 22793* (A). Kansas: Riley County, *Norton 502* (GH); Neodesha, *Palmer 20823* (A); Ellsworth, *Palmer 21281* (A). Oklahoma: Ft. Sill, *Clemens 11541* (GH); Norman, *Bruner*, 1924 (A); Oklahoma City, *Slavin 421* (A); Purcell, *Stevens 137* (GH); Sulphur, *Merrill*, 1935 (A). Texas: Travis County, *Warnock 46090* (GH); Dallas. *Reverchon*, 1874 (GH).

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